Human Immunology 80 (2019) 983–989

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Development of data-driven models for the flow cytometric crossmatch

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ARTICLE INFO

Keywords:
HLA
Data-driven modeling
Virtual crossmatch
Organ allocation

ABSTRACT

HLA laboratories use virtual crossmatching (VXM) to predict recipient and donor compatibility using HLA antibody data and donor HLA type. Increasingly, transplant centers are utilizing VXM as the final compatibility determination prior to transplant. However, the VXM interpretation is based on HLA experience of individual transplant centers. This study developed data-driven algorithms that predicted flow cytometric crossmatch (FCXM) outcomes using HLA antibody mean fluorescent intensity (MFI) data and donor HLA typing without the need for human interpretation. Two algorithms were evaluated; an MFI Optimal-Threshold model and a Least-Squares-Fitting model. The Optimal-Threshold model correctly determined between 81.5% and 85.5% of T or B-cell responses. A class I antibody MFI threshold of 4670 was optimal for predicting T-cell response while an antibody MFI threshold of 6180 was optimal for predicting B-cell responses. HLA class I antibodies had a 1.47-fold greater influence on FCXM outcomes than class II antibodies. HLA-B antibodies influenced T and B-cell responses more than HLA-A or -C (-B > -A > -C). The Least-Squares-Fitting model increased accuracy to 94.1% and 88.8% for T and B-cell responses, respectively. The algorithms described here provide enhanced FCXM prediction and novel insights into the influence of specific HLA antibodies on the crossmatch outcome.

1. Introduction

Prolonging organ viability has been a long-term goal within the transplant community for numerous years. There have been many successful efforts towards improving rates of acute organ rejection, particularly for kidneys [1]. Introduction of the complement dependent cytotoxic (CDC) crossmatch and improve immunosuppression enabled better risk stratification of kidney recipients and reduced rates of organ rejection [1–3]. However, there were cases of false-negative CDC crossmatch in which organ rejection occurred as well as technologic advances which lead to the development of the flow cytometric crossmatch (FCXM) [4–7]. FCXM has been shown to be more sensitive than CDC [8,9]. With the advent of solid phase immunoassays (SPI), human leukocyte antigen (HLA) laboratories have a highly-sensitive tool to detect HLA antibodies [10,11]. Laboratories now routinely list "unacceptable" HLA antigens based off SPI testing. However, Kerman et al. demonstrated the ineffectiveness of FCXM to reduce one-year organ rejection [4]. Use of SPI testing and subsequent patient sensitization calculator greatly reduced the rate of organ refusal due to positive crossmatches [12].

While FCXM is still considered the "gold-standard" most transplant centers are combining SPI testing and donor HLA typing transplant centers to perform virtual crossmatching (VXM). VXM can be applied across all organ and tissue transplantation. The goal of VXM is to further decrease the rate of organ refusal due to positive crossmatches and reduce cold ischemia time. Increasingly, transplant centers are relying on VXM as the final compatibility test for non-sensitized patients [11,13,14]. A study by Johnson et al. used SPI as the final allocation decision for renal transplantation and found no difference in acute rejection or 5-year graft survival between FCXM positive and negative recipients. Importantly, there were positive FCXM in the absence of DSA. The positive FCXM cohort had higher risk for rejection due to a number of variables including type of donor, sensitization rate, duration of dialysis, and PRA score [13]. Additionally, other groups have found the accuracy for VXM to range between 89% and 97% of cases [15–17]. The accuracy of VXM is highly dependent on SPI results and can be less accurate for highly-sensitized (cPRA > 80%) patients [18]. The disparity between highly-sensitized and other recipients has led to

Abbreviations: HLA, human leukocyte antigen; SAB, single antigen bead; MFI, mean fluorescent intensity; DSA, donor-specific antibody; VXM, virtual crossmatch; FCXM, flow cytometric crossmatch; DDA, data-driven algorithm; MCS, median channel shift; SPI, solid phase immunoassay; nlS, normalized improvement score

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https://doi.org/10.1016/j.humimm.2019.09.004
Received 28 June 2019; Received in revised form 13 August 2019; Accepted 5 September 2019
Available online 14 September 2019

increased offered rejections in as much as 16% of cases [11].

The known limitations of VXM included technical issues related to SPI testing and HLA genotype prediction. These limitations have made accurate VXM prediction of cell-based crossmatches has proven challenging [19,20]. Currently, the best technique to improve FCXM prediction is by listing unacceptable HLA antigen based on transplant center-specific MFI thresholds. Using varying MFI thresholds increases VXM prediction to approximately 96% [17]. The majority of previous attempts at predicting FCXM outcomes have relied on the summation of donor-specific antibody (DSA) MFI values from SPI testing [21,22]. However, SPI only correlates with approximately 85% of FCXM results [23]. Additionally, Ellis et al demonstrated a 90% sensitivity for T cell mean channel shifts (MCS) prediction and 57% sensitivity for B cell MCS prediction [11] using MFI values. Other reports utilizing a similar mathematical approach have yielded a prediction accuracy ranging from 79% and 90% [18,24–27]. This simplistic approach to predicting FCXM results fails to incorporate the true complexity of cell-based crossmatches, the relative weights of particular DSA, and may introduce human biases (confirmation or recency) [28].

A drawback of VXM is the reliance on not only SPI MFI values but also human prediction of the impact of various DSA on FCXM outcomes. Cognitive biases are a well-established phenomenon in human learning to promote fast learning [29–31]. Human bias impacts how an individual would interpret the same day over extended periods of time and often manifests as either conformation bias or recency effect. To reduce VXM dependency on human prediction, data-driven modeling algorithms can be employed to predict the likely FCXM outcome based entirely on computer-based learning from empirical evidence, providing an unbiased approach to modeling. Data-driven modeling of biologic data, including immunologic studies and transplant rejection, has been proven to be highly accurate [32,33]. Algorithms built from data-driven models are easily adapted to changing technology and an enhanced understanding of the biologic system.

In this study, we applied two different data-driven modeling approaches to predict the FCXM outcome for T and B cells. Each model was evaluated for accurate prediction of T and B cell outcomes. The models were utilized to examine the relative importance of HLA loci as well as individual HLA allele groups on FCXM outcome. Importantly, this study represents the first application of data-driven modeling to predict FCXM outcome. The models presented here can be applied to other clinical settings where DSA can impact outcomes such as hematopoietic cell engraftment.

2. Material and methods

2.1. Datasets

Only samples that had SAB class I and II MFI data were included in the study. The data (April 2016-August 2018) consisted of 303 FCXM outcomes and 252 serum samples of HLA locus-specific MFI data and FCXM outcomes (Fig. 1). Normalized MFI data was compiled in a donor-specific manner using available donor HLA typing information excluding HLA-DPB1 and -DPA1. Donor serologic HLA typing was determined using historic SSO HLA typing data or high-resolution HLA typing. When available DSA MFI data was compiled using high-resolution HLA typing, if high-resolution HLA type was not represented on a bead in the solid phase assay or if high-resolution HLA typing was unavailable the highest DSA MFI bead was used. All SAB data was generated using OneLambda SAB assay and performed according to the manufacturer's instructions so some modifications. Sera prior to August 2017 were not treated. Sera after the above date were treated with EDTA prior to the OneLambda SAB assay. UNC clinical validation demonstrated that EDTA treatment did not significantly alter the MFI data in the majority of samples (UNC HLA laboratory unpublished data). Consistent with published reports, EDTA pre-treatment enhanced detection of DSA in prozone samples [34–36]. FCXM data were extracted

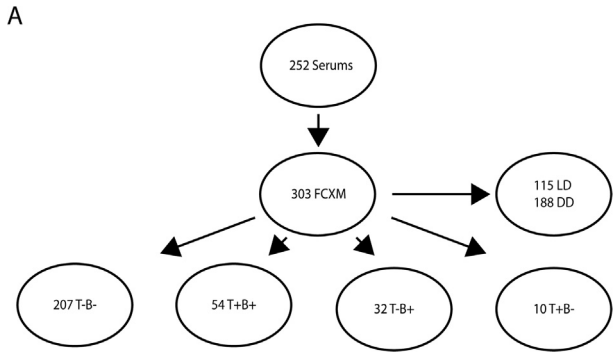

Fig. 1. Overview of Study Characteristics. (A) 2016–2018 individual HLA DSA data and flow cytometric crossmatch outcomes. All flow cytometric crossmatches were performed using pronase-treated lymphocytes (see Section 2).

from the HLA laboratory information system (HistoTrac, SystemLink). All FCXM were performed using pronase treated of lymphocytes and MCS cutoffs determined using normal human serum according to established laboratory practices at the time of FCXM. MCS cutoff values were the same across all donor types and are determined/validated quarterly. All available FCXM were used in the study regardless of organ type. Deceased donors lymphocytes were isolated from peripheral blood. For the Supplemental data, false negative FCXM (not predicted results) were defined as negative FCXM in presence of DSA greater than 4500 MFI. False positive FCXM (not predicted results) were defined as positive FCXM with no single DSA greater than 200 MFI. True positive FCXM results were defined positive FCXM with a single DSA $>$ 1000 MFI. True negative FCXM were defined as negative FCXM with a single DSA less than 1000 MFI. Fig. 1 displays the breakdown of the FCXM used in the study. This study was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill.

2.2. Mathematical modeling

Two data-driven modeling techniques were employed to perform VXM prediction. The first Optimal-Threshold method formalizes what is already done by HLA experts by considering the summed effect of MFI data. The second Least-Squares-Fitting method predicts the actual FCXM outcome for T and B cells.

The Optimal-Threshold method is based on assigning a positive or negative crossmatch if the summation of the relevant MFI data is below or above an assigned threshold, respectively. The optimal threshold to use is chosen so that the maximum number of data points are correctly differentiated, thus maximizing the number of points that are correctly accepted and correctly rejected. A MatLab (R2017a) script was developed to test thresholds in increments of 10.

The Least-Squares-fitting method creates a weighted sum of the DSA MFI data that best predicts the FCXM outcome for T and B cells. The method was applied separately to fit the HLA class I allele group antibodies to predict either T cells or B cells and fit all the allele group antibodies (HLA class I and II) to predict B cells. Specifically, let $\hat{T}_j$ be the predicted FCXM outcome for the T cells of the j^{th} patient. We take

$$\hat{T}_j = \sum_{i=1}^{N} \beta_i x_{ij}$$

where the β_i are the weights of the N_I class I alleles with x_{ij} the MFI values of patient j corresponding to the i^{th} allele. Using MatLab's built in fminunc routine, the β_i were found such that they locally minimize the square of the distance between the predicted T cell outcome, $\hat{T}_j$, and the true T cell outcome, T_j, from the FCXM summed over all N_p patients,

$$d^2 = \sum_{j=1}^{N_p} |\hat{T}_j - T_j|^2.$$

Notice that the β_i are the same for each patient. Since multiple local minima exist, the minimization routine was repeated 1000 times from randomly chosen starting values for the β_i and the results averaged to obtain a set of significant fitting parameters, shown in Fig. 3B. A similar procedure was used to find fitting parameters to predict the B cells using only the class I HLA data (Fig. 3B), and to predict the B cell data using both the class I and class II HLA data (Fig. 3C).

In addition to the percent accuracy, the performance of the data-driven methods was quantified with a normalized improvement score (nIS), defined as the fraction of improvement over the baseline of accepting or rejecting all patients. Taking the maximum percent accuracy between MFI threshold of zero and infinity, B%, the normalized improvement score is the fraction of the distance to 100% the data-driven percent accuracy achieved, D%, normalized by the available improvement,

$$\mathrm{nIS} = \frac{\mathrm{D} - \mathrm{B}}{100 - \mathrm{B}}.$$

In this way, an improvement score of 1 corresponds to a perfect method and higher nIS correspond to better methods even when the percent accuracy is lower.

3. Results

3.1. Data used in the study

The data consisted of 252 serum samples with their individual DSA HLA class I loci MFI and class II loci MFI data were compiled in connection with corresponding 303 FCXM outcomes. The 303 FCXM consisted of 115 living-donor and 188 deceased donor FCXM (Fig. 1A). Overall FCXM outcomes from the data are shown in Fig. 1. There were 207 negative FCXM (68.3%) (T and B cell negative), 54 FCXM were T and B cell positive (17.8%), 32 were T cell negative and B cell positive (10.6%), and 10 were T cell positive and B cell negative (3.3%) (Fig. 1A).

3.2. Optimal threshold model prediction of FCXM outcomes

The first mathematical approach that determines the optimal MFI threshold that yields the highest level of prediction accuracy (see Section 2). This approach is based on the summation of the DSA HLA class I and/or class II MFI data and is similar to current VXM practices. Additionally, to compare the performance of different algorithms and within/across data sets, a normalized improvement score was determined (see Section 2). The higher the nIS the better the performance of the algorithm. Two MFI thresholds were instituted as controls. A DSA MFI threshold of 0 would cause a prediction of ALL recipient and donor pairs to be positive. In contrast, a DSA MFI threshold of infinity would cause a prediction of ALL recipient and donor pairs to be negative. The low algorithm performance, based on nIS, confirms class II data is incapable of predicting T cell FCXM outcomes. Consistent with T cell lack of HLA class II expression.

Both optimal thresholds based on class subdivision as well as further subdivision into HLA-A, HLA-B, and HLA-C were evaluated. These results are summarized here, with detailed in Table 1. Statements regarding false negative, false positive, true negative, and true positive were made comparing physical FCXM results to the algorithm prediction. The DSA HLA class I data predicted 85.5% (259/303) of FCXM T cell outcomes with a threshold of 4670, for a nIS of 0.313 (Table 1). An MFI threshold of 9740 was found for DSA class II prediction of FCXM T cell results, for a nIS of 0.047. Using both class I and class II, an MFI threshold of 6180 predicted 81.5% of B cell outcomes, for a nIS of 0.345. HLA-B antibodies affected the accuracy of T cell prediction 1.87-fold more than HLA-A and 5.37-fold more than HLA-C. An optimal DSA MFI thresholds of 2240, 2110, and 8230 were identified for HLA-B, -A, and –C antibodies, respectively (Fig. 2B, Table 1). In contrast, MFI thresholds of 3610 and 950 were optimal for HLA-DRB1 and -DQ, respectively (Table 1). The left panel of Fig. 2C illustrates which values were correctly predicted with these subdivisions using T cell FCXM MCS. Of note, the black dots represent likely false positive physical FCXM results since there are no class I DSA detected; however, the algorithm predicted those FCXM to be negative, consistent with the biology (see Supplemental Table 1). Using the threshold model prediction of the T cell FCXM outcome correlated with the ability to correctly predict the B cell outcome as well (Fig. 2C, right panel). However, using the nIS number, T cell prediction performed 1.2-fold better than B cell prediction (Table 1).

3.3. Least-squares model prediction of FCXM outcomes

Since the Optimal Threshold model yielded between 85.5% (T cells) and 81.5% (B cells) accuracy, there was clear evidence that prediction improvement was possible. The next modeling approach developed utilized least-squares fitting of a weighted average (detailed description in). Briefly, this method attempts to minimize the distance between the predicted FCXM outcome and the true FCXM outcome by determining relative weights (or importance) of antibodies against particularly HLA allele groups. Although the majority of the data set are T cell and B cell negative (Fig. 1) the Least-Squares approach determines the relative importance of all DSA on the FCXM median channel shift (MCS) outcome thus the negative qualitative FCXM results don't impact the quantitative results from which the algorithm attempts to minimize the distance. The Least-Squares approach yielded an accuracy of 94.1% and 88.8% for FCXM T and B cell outcomes, respectively (Table 2). Importantly, if suspected false negative and false positive FCXM are removed from the analysis the accuracy of the Least Squares model increased to 97.9% (T cells) and 90.0% (B cells) (Supplemental Table 2). The individual data points for this calculation are shown in Fig. 3A; the distance of the points from the solid black line is a measure of the error of the prediction. The overall improvement of the least squares model was 2.30-fold for T cells compared to the threshold algorithm (Table 2). Using the least squares approach, the accuracy of predicting B cell outcomes was increased 1.77-fold. While HLA class I antibodies alone correctly determined 87.1% of B cell responses, the inclusion of HLA class II antibody data improved the prediction 1.12-fold (Fig. 3A; Table 2). The fit coefficients, relative importance, for each HLA allele group are shown in Fig. 3B (class I only) and Fig. 3C (class I and II). Larger values indicate a stronger correlation to the T or B cell outcome while a larger magnitude below zero indicates a stronger negative correlation to the T or B cell outcome. Prediction of T and B cell outcomes was most affected by the presence of antibodies against HLA-C14 and HLA-B81.

In general, HLA class I antibodies had a similar effect on T and B cells (Fig. 3B). There were a few HLA groups where their impact on T and B cells were not consistent; HLA-A33, A69, B37, B38, B41, B50, B81, and C12. Some of the HLA groups listed above had greater influence on T cells compared to B cells or vice versa. Comparing the fit parameters to predict B cell outcome from Fig. 3B (red) with those in Fig. 3C, the class I values have similar relative importance. The importance of the class II antibodies is noticeably less, resulting only in a 1.12-fold improvement in prediction of B cell outcome. Individually HLA class II antibodies played a negligible role in B cell prediction. Other observations include that eleven of the fifteen (73.3%) HLA-C beads were found to have a negative influence on FCXM prediction compared to only 16.7% (3/18) of HLA-A or 22.6% (7/31) of HLA-B beads.

Least-Squares determination of relative importance would be affected by the number of occurrences of a particular HLA allele group. To ensure the correct interpretation of the relative importance data, the number of occurrences of each HLA allele group was determined. Several were present once (A69, B37, B41) in our dataset (Fig. 3D). Thus, the determination of the true importance of those HLA allele

Table 1
Accuracy and Predictive value of Optimal-Threshold modeling of FCXM. Cl I, HLA class I; Cl II, HLA class II; MFI, mean fluorescent intensity; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity; nIS, normalized improvement score.

			MFI Threshold	True Positive	True Negative	False Positive	False Negative	Total Correct	Percent Correct	NPV	PPV	Sens	Spec	nIS
Cl I/Cl II	→	T-cell	0	64	0	239	0	64	21.1%					
Cl I/Cl II	→	T-cell	∞	0	239	0	64	241	78.9%					
Cl I/Cl II	→	B-cell	0	86	0	217	0	86	28.4%					
Cl I/Cl II	→	B-cell	∞	0	217	0	86	219	72.3%					
Cl I	→	T-cell	4670	36	223	16	28	259	85.5%	88.8%	69.2%	56.3%	93.3%	0.313
Cl II	→	T-cell	9740	14	228	13	50	242	79.9%	N/A	N/A	N/A	N/A	0.047
A	→	T-cell	2110	24	229	10	40	253	83.5%	85.1%	70.6%	37.5%	95.8%	0.218
B	→	T-cell	2240	33	232	7	31	265	87.5%	88.2%	82.5%	51.6%	97.1%	0.408
C	→	T-cell	8230	6	238	1	58	244	80.5%	80.4%	85.7%	9.4%	99.6%	0.076
Cl I & Cl II	→	B-cell	6180	51	198	19	36	247	81.5%	84.9%	70.8%	59.3%	90.3%	0.345
DRB1	→	B-cell	3610	24	213	4	62	237	78.2%	77.5%	85.7%	27.9%	98.2%	0.213
DQ	→	B-cell	950	21	201	16	65	222	73.3%	75.6%	56.8%	24.4%	92.6%	0.036

groups is difficult to assess. The HLA allele groups found to influence the FCXM prediction were present in greater than a single case in our data set; HLA-C14 was present in 3, A26 was in 9, C12 in 20, B81 in 3 cases (Fig. 3D).

4. Discussion

The data described here demonstrate the effective development of an algorithmic approach to determining FCXM results without human bias or intervention. Many HLA laboratories use VXM to predict recipient and donor compatibility prior to performing FCXM; however, the process is labor intensive and recency effect [37] can influence interpretation. Understanding the accuracy of VXM determination and the complex relationship between DSA MFIs and HLA antigens expressed by the donors is increasingly important. It is important to note that the two algorithms were employed without prior knowledge of HLA, transplant biology, or direct human influence. The threshold model accuracy is consistent with previous reports on modeling FCXM outcomes (Fig. 2, Table 1) from SAB data however the Least-Squares method proved the most accurate for T and B cell FCXM prediction (Fig. 3, Table 2) [16,17,38,39].

The accuracy of both modeling approaches is dependent on the MFI values of DSA from the SAB assay. The SAB assay has well-established phenomenon of increased reactivity including denatured HLA antigens, increased protein concentrations on the solid phase beads, and variability [9,10,40–42]. Even with reports of CVs of 20–40% for the SAB assay depending on assay and HLA locus, the models presented here still provide accurate results. Additionally, the models predicted our current understanding of biology (i.e. HLA class II is absent on T cells and ineffective at T cell prediction) without human intervention or bias.

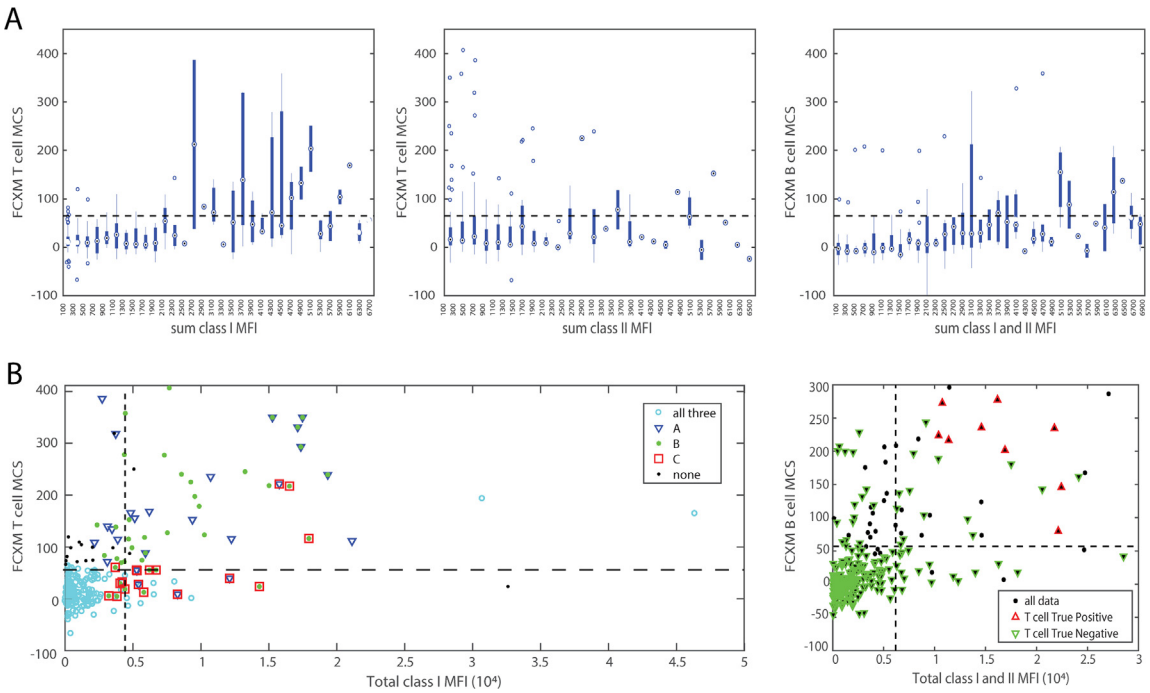

Fig. 2. Optimal DSA threshold determination and FCXM outcomes. Optimal DSA thresholds (vertical dotted lines) were determined using a summation of HLA DSA data based on mean fluorescent intensity (MFI). Referring to the *predicted* FCXM outcomes; FN, false negative; TP, true positive; TN, true negative; FP, false positive. (A) Correlation between summations of HLA class I (left) or class II (middle) DSA on T cell median channel shift (MCS). Correlation of summation of HLA class I and II DSA and B cell MCS (right). (B) Correlation of individual HLA loci DSA with T cell MCS. Open circle, all three class I loci correctly predicted T cell outcome; Triangle, HLA-A correctly predicted; Dot, HLA-B correctly predicted; Square, HLA-C correctly predicted; Point, samples with no class I DSA correctly predicted. Right plot – Impact of T cell MCS prediction of B cell MCS. All data, regardless of T cell prediction, are presented in black dots. Upright triangle, data with T cell true positive prediction; Upside down triangle, data with T cell true negative prediction.

Table 2
Accuracy and Predictive value of Least-Squares modeling of FCXM. Cl I, HLA class I; Cl II, HLA class II; MFI, mean fluorescent intensity; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity; nIS, normalized improvement score.

			True Positive	True Negative	False Positive	False Negative	Total Correct	Percent Correct	NPV	PPV	Sens	Spec	nIS
Cl I	→	T-cell	51	234	5	13	285	94.1%	94.7%	91.1%	79.7%	97.9%	0.720
Cl I	→	B-cell	55	209	8	31	264	87.1%	87.1%	87.3%	64.0%	96.3%	0.554
Cl I & Cl II	→	B-cell	63	206	11	23	269	88.8%	90.0%	85.1%	73.3%	94.9%	0.618

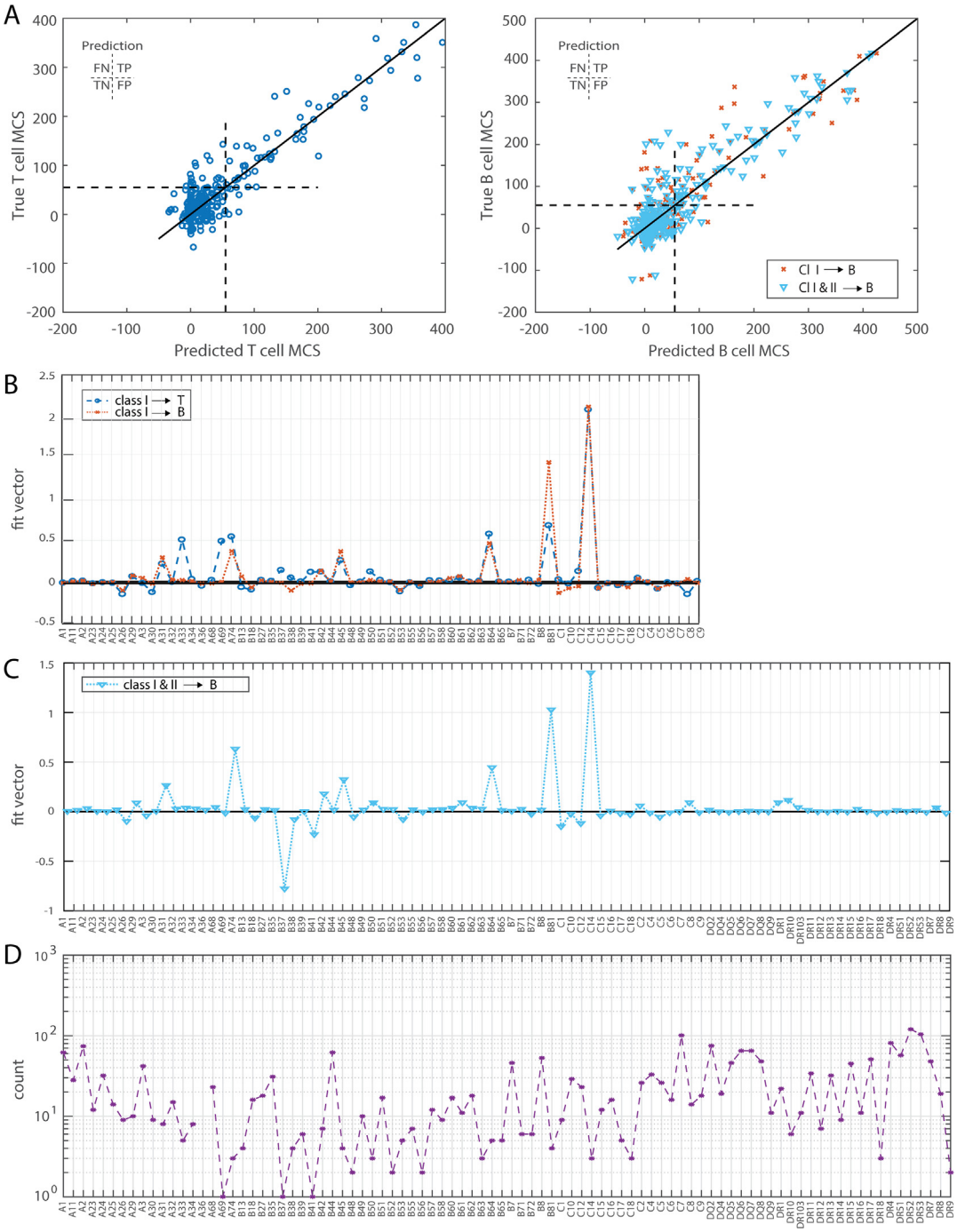

Fig. 3. Least-Squares modeling improves T and B cell FCXM prediction. (A) True T cell (left) and B cell (right) FCXM results compared to the predicted T cell MCS (left) and predicted B cell MCS (right). Dotted lines represent the approximate real-world FCXM cutoff (Section 2). For B cell prediction class I (dots) and class I & class II (triangles) were used. FN, false negative; TP, true positive; TN, true negative; FP, false positive. (B) Fit vector values (relative importance) for HLA class I DSA on T cell (blue dots) and B cell (red stars) FCXM prediction. (C) Fit vector values (relative importance) for HLA class I & II DSA on B cell FCXM prediction. (D) Count of donor HLA antigen groups present in the study. HLA antigens (A36) without a point indicates that antigen group was present in the study but had an MFI value of zero. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The current HLA laboratory practice of using MFIs as a relative gauge for predicting FCXM results. The Optimal Threshold model determined unbiased ideal MFI thresholds of 2110, 2240, 7300, and 6180 for HLA-A DSA, -B DSA, class I DSA, and class I and II DSA, respectively (Tables 1 and 2). While these MFI values are consistent with current HLA laboratory experiences [17,43], the models were not instruct on such experiences further demonstrated the utility of unbiased modeling for VXM. In contrast, the optimal threshold for DQ antibodies was considerably lower at 950 MFI. The lower MFI threshold for DQ antibodies is most likely related to the relative lack of DQ sensitization compared to the other HLA loci among our data set. The mean MFI for DQ antibodies was 763 compared to 923, 1102, 869, and 1674 for HLA-A, -B, -C, and -DRB1 (data not shown).

Many transplant centers use an MFI range of 3000–5000 to list unacceptable HLA antigens in UNOS. While the results from the Optimal-threshold algorithm are consistent with that laboratory practice, the data illustrate the differences in DSA against individual HLA loci (Table 1). This observation supports the use of HLA loci specific DSA cutoffs for listing of unacceptable HLA antigens. Similar data has been shown for DP DSA, which often require very high MFI values to promote positive B cell FCXM outcomes [44,45]. Thus, a practical application of the Optimal Threshold model is the determination of HLA locus specific unacceptable MFI ranges. Importantly, the algorithm correctly predicts the inability of class II DSA to determine T cell outcomes (Table 1). Collectively, these data can help inform transplant centers on the impact MFI thresholds have on the prediction of FCXM outcome and the subsequent transplant risk.

The threshold model demonstrates the importance of HLA-B DSA over HLA-A or HLA-C on T cell FCXM outcomes (Table 1, Fig. 2C). Multiple studies have demonstrated that HLA-B and HLA-A have the highest relative expression on T and B cells compared to HLA-C using RNASeq, flow cytometry, or mass spectrometry [46,47]. Consistent with similar expression of HLA-A and -B, both HLA loci had similar DSA thresholds (Table 1). While the algorithm determined that DSA to HLA-C14 and B81 were critical to T and B cell predictions, DSA to HLA-B37 or B41 were the least critical to B cell prediction. Consistent with the increased impact of C14 antibodies, C14 has been shown to have the highest expression compared to other HLA-C antigens [47–50]. The increased number of HLA-C antibodies identified as less important for FCXM prediction correlates with the over-reactivity of the HLA-C beads in the SAB assay. Extremely low cross-reactivity was present in the relative importance determination (Fig. 3C). For example, B21 CREG contains B50 and B49; however, only B50 antibodies had a significant influence on FCXM prediction. Additionally, the B12 CREG contains B44 and B45; however, only B45 antibodies had a positive influence on FCXM prediction. There are numerous additional examples of observation. Only DSA to HLA-DR1, -DR10, -DR103 DSA demonstrated any appreciable impact on B cell MCS (Fig. 3D). Since those HLA antigen groups have no association with DRB3/4/5 it suggests an increased importance for DSA in the absence of DRB3/4/5. However, collectively HLA class II DSA increased the prediction accuracy from 87.1 to 88.8%, increasing the negative predictive value (NPV) to 90.0% (from 87.1%) with only a 2.5% reduction in the positive predictive value (PPV) (Table 2).

Understanding of HLA biology for recipient and donor compatibility is vital for organ allocation systems. Both algorithms provide insights into the complex HLA biology in an unbiased fashion that are consistent with laboratory experience. For example, HLA-C DSA requires a higher MFI compared to HLA-B and HLA-A DSA to promote a positive FCXM (Table 1). Importantly, these observations by the algorithms are despite the fact that the physical FCXM is a somewhat flawed reference method with known issues, including pronase treatment of lymphocytes, false positive T cell FCXM, and application of universal MCS cutoffs [51–53]. While our FCXM outcomes are determined using universal MCS cutoffs, clinical validation studies performed biannually have determined that MCS cutoff between living and deceased donors to be equivalent. As evidence of the benefit of the modeling approach over physical FCXM, if suspected false negative or positive FCXM were removed from the analysis the accuracy of both models increased while the MFI threshold remained relatively stable (Supplemental data). This observation suggests the models are correctly predicting true immunologic compatibility and are not influenced by autoantibodies, cryptic epitopes, or drug interferences as FCXM can be influenced [51,54,55].

A deficiency in both models is the lack of incorporation of other important biologic factors that can influence FCXM outcomes as well the need for more HLA class II antibody only data. These factors include variability in donor and organ HLA expression, variability in SAB assays, shared epitope analysis, and HLA antibody avidity. Another important limitation is the need for an independent data cohort validation, more positive FCXM, and HLA-DP antibody assessment. The timely nature of organ allocation makes incorporation of donor-specific HLA expression currently impractical, however, application of generic HLA locus-specific expression data such as those generated from existing RNASeq data [46,56,57] could be used for algorithm improvement in the future. Incorporation of HLA antibody avidity is feasible since it could be determined while patients are on the waitlist. Even without the inclusion of these parameters the algorithm was able to correctly predict 94.1% and 88.8% of T and B cell cases, respectively (Table 2). Future studies are planned to investigate the incorporation of the above biologic elements into the algorithms as well as enhance the model to use epitope-based antibody profiling for recipient and donor pairs.

Conflict of interest

Dr. Weimer reports personal fees from OneLambda, personal fees and non-financial support from CareDx, outside the submitted work; All other authors have no relevant disclosures.

Acknowledgement

The authors would like to thank the UNC HLA laboratory for their efforts in HLA antibody data generation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.09.004.

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